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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANTS	:	JÜRGEN HESCHELER
SERIAL NO.	:	TO BE ASSIGNED
FILED	:	HEREWITH
FOR	:	FLUORESCENT PROTEINS AS CELL-TYPE SPECIFIC REPORTERS
ART UNIT	:	1632
EXAMINER	:	J. Woitach

February 28, 2002

Hon. Commissioner of Patents
Washington, D.C. 20231

PRELIMINARY AMENDMENT

SIR:

Prior to examination, please amend the above-identified application as follows:

IN THE SPECIFICATION:

Insert as the first paragraph of the specification the following new paragraph:

--This application is a continuation of U.S. Serial No. 09/446,717, filed on April 13, 2000, now pending, which is, in turn, a 371 of PCT/EP98/03988, filed on June 30, 1998. Priority of both applications is claimed.--

IN THE CLAIMS:

Cancel the original claims and substitute the following new claims:

15. A cell culture exhibiting cell-type specific or development-specific expression of a non-cell-damaging fluorescent protein due to activation of a cell-specific or development specific transcription factor at a certain point of differentiation in differentiating cells, said cell culture consisting of embryoid bodies formed by aggregates of embryonic stem (ES) cells of mice obtained by the hanging drop method and stably transfected with a DNA construct comprising:

a) a DNA sequence coding for said non-cell damaging fluorescent protein; and

b) a promoter operably linked with said DNA sequence, said promoter selected from a cell-dependent promoter, a development-dependent promoter, and a combination of a cell-dependent and a development-dependent promoter;

said DNA construct being integrated in the native DNA.

16. The cell culture according to claim 15, wherein said non-cell-damaging fluorescent protein is selected from Green Fluorescent Protein (GFP), Red Fluorescent Protein and Blue Fluorescent Protein.

17. The cell culture according to claim 15, wherein said promoter is a promoter specific for heart cells, neurons, glia cells, hematopoietic cells, endothelial cells, smooth muscle cells, skeletal muscle cells, cartilage cells, fibroblasts or epithelial cells.

18. The cell culture according to claim 17, wherein said promoter is selected from Nkx-2.5, human α-actin and MLC-2V promoters.

19. The cell culture according to claim 18, wherein said promoter is the heart-specific human α-actin promoter.

20. The cell culture according to claim 15, wherein said DNA construct includes further functional DNA elements.

21. The cell culture according to claim 20, wherein said further functional DNA elements are selected from enhancer elements, selectable marker genes, or combinations of enhancer elements and selectable marker genes.

22. The cell cultures according to claim 15, wherein said DNA construct is the plasmid pCX-(a-act)GFP-Neo (DSM 11633).

23. A method for preparing a cell culture according to claim 15, comprising:

a) introducing a DNA construct as defined in claim 15 in starting ES cells of mice;

b) screening for stably transfected ES cells; and

c) establishing embryoid bodies from said stably transfected ES cells in accordance with the hanging drop method.

24. The method according to claim 23, wherein said introducing is effected by electroporation.

25. The method according to claim 23, further comprising the culturing of said stably transfected ES cells in vitro.

26. A method for the toxicological examination of substances, comprising adding the substances to the cell cultures according to claim 15 and examining the toxicological effects of said substances on the cell cultures, using fluorimetric methods.

27. A method for producing a transgenic mouse exhibiting cell-type specific or development specific expression of a non-cell-damaging fluorescent protein, comprising:

a) injecting ES cells according to claim 15 into blastocysts of a mouse;

b) transferring the blastocysts into a surrogate mother; and

c) recovering said transgenic mouse from said surrogate mother.

28. A transgenic mouse obtainable by the method according to claim 27.

29. A method of using the mouse according to claim 28 for examining stages of development of cells, comprising examining the correspondingly marked cells of said mouse in vitro using fluorimetric methods.

30. A method for determining whether a substance influences the differentiation of cells comprising adding the substance to cell cultures according to claim 15, and then at various times thereafter determining the area of cells expressing fluorescent protein, and then using the determinations to give an indication whether the substance influences the differentiation of the cells.

31. A method according to claim 30, which is for determining whether a substance influences the differentiation of heart cells, said method comprising adding the substance to cell cultures according to claim 15, and then at various times thereafter determining the area of heart cells expressing fluorescent protein, and then using the determinations to give an indication whether the substance influences the differentiation of the heart cells.

REMARKS

This application is a continuation of U.S. Serial No. 09/446,717 (hereinafter "the parent"), which is still pending. Claims 15-29 are identical to claims 15 and 17-30 pending in the parent at the time that prosecution therein was terminated. Claims 30 and 31 are new, and are

drawn to a different method, which is based on the paragraph bridging pages 6-7 of the specification. The presence of these new claims should preclude a first action final rejection. In the event that the Examiner believes claims 30 and 31 constitute a separate patentable invention from the other claims and intends to require restriction, then Applicant respectfully requests that a written restriction requirement be issued.

Early and favorable action is earnestly solicited.

Respectfully submitted,

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